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Thiol and Cardiovascular Risk Factor Status in a Male Northern Irish Population

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Abstract: *Objectives:* Raised plasma homocysteine is a risk factor for cardiovascular disease (CVD). Cysteine has also been associated with CVD risk. In this study, we investigated the association between known CVD risk factors, dietary factors, and total plasma cysteine, cysteinyl-glycine, and homocysteine.

Methods: The study group was 765 male workers aged between 30–49 years. The dietary habits of the subjects were recorded using a food frequency questionnaire. Body mass index (BMI), smoking status, and blood pressure were assessed, and fasting blood samples were taken for analysis of serum concentrations of vitamins, lipids, total plasma cysteine, cysteinyl-glycine, and homocysteine, and genotyping for the methylenetetrahydrofolate reductase (MTHFR) polymorphism.

Results: In multivariable analyses, cysteine was significantly positively associated with age and negatively associated with serum vitamin B_{12} and serum vitamin B_6, while cysteinyl-glycine was significantly positively associated with BMI. Homocysteine (tHcy) was significantly negatively associated with serum folate, serum vitamin B_{12}, and fruit and vegetable intake, and also depended on the MTHFR 677C > T genotype.

Conclusions: Our data show a significant relationship between age, serum levels of B-vitamins and cysteine, and BMI and cysteinyl-glycine. In agreement with other studies, we also confirm an association between tHcy, serum folate and vitamin B_{12}, MTHFR genotype, and fruit and vegetable intake. Further investigation into the role of these thiols and their determinants in CVD is required.

Key words: Thiols, homocysteine, B vitamins, cardiovascular risk factors, MTHFR

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Introduction

High total plasma homocysteine (tHcy) concentration (hyperhomocysteinemia) is an independent risk factor for cardiovascular disease (CVD) [1, 2]. Cysteine ($C_3H_7NO_2S$) is another sulfhydryl-containing amino acid that is structurally similar to homocysteine. The body can chemically transform homocysteine to cysteine via the trans-sulfuration pathway in a two-step process catalyzed by two vitamin B_6-dependent enzymes, cystathionine β-synthase and cystathionase. The association between total plasma cysteine (tCys) and CVD has not been investigated to the same extent as tHcy, however, some studies have shown raised tCys in patients with vascular diseases [3–6], although not all studies have demonstrated this association [7, 8]. Total cysteinyl-glycine (tCys-gly) has not been studied in relation to CVD risk, but is metabolically linked to tHcy and tCys, with which it can be quantified in the same chromatography assay [9]. Alterations in thiol status have been observed in a range of other diseases, including pre-eclampsia [10], rheumatoid arthritis [11], type 2 diabetes [12], and end-stage renal disease [13].

CVD is a major public health issue in Northern Ireland. tCys and tHcy appear to be indicators of risk, yet, apart from serum B vitamins and tHcy, little is known about the association between plasma thiol status, nutritional status and intake, and other CVD risk factors. It has been suggested that the association between elevated tHcy and CVD risk is indirectly mediated through classic CVD risk factors such as smoking and hypertension [14, 15] (i.e. that hyperhomocysteinemia is a risk marker rather than a risk factor). In this study the associations between thiol concentrations, dietary intake and serum B-vitamins, and CVD risk factors were investigated.

Subjects and Methods

The study was approved by the Research Ethics Committee of the Faculty of Medicine, Queen's University Belfast.

Subjects and clinic procedure

Participants were male volunteers aged 30–49 years employed by a single, large, Belfast-based company and included all grades of staff (i.e. manual, clerical, administrative, and executive). Employees within the correct age group, identified by the company Occupational Health Unit, were contacted by letter. Approximately 40% responded (n = 765) and they were asked to attend the Occupational Health Unit clinic at 7.30 am. Each subject signed a consent form after receiving an information sheet which explained the purpose of the research, the examination they would undergo, and emphasized that results would be kept confidential. They were asked to complete a previously validated food frequency questionnaire (FFQ) [16]. The questionnaire was checked and, if required, assistance given in its completion by a nutritionist; a brief medical history was taken, and height and weight were measured. Demographic data and smoking status (smoker or non-smoker; time since stopping smoking; number of cigarettes smoked per day) were recorded. Blood pressure was recorded using a Spengler automated sphygmomanometer on the right arm and a fasting venous blood sample was then taken from the left arm. An EDTA-treated sample was used to provide plasma for tHcy, tCys, and tCys-gly analysis and a clotted sample provided serum for vitamin analysis. Plasma was separated from the EDTA blood samples within 15 minutes of venipuncture at 4°C, while the serum samples were kept in the dark and returned to the laboratory for processing. Samples were aliquotted as required. All samples were stored at –80°C until analysis. Samples were collected between November 1994 and May 1995, and all sample analysis was completed by May 1997.

Blood measurements

TCys, tCys-gly, and tHcy (both free and protein-bound) were assayed by high-performance liquid chromatography (HPLC) according to the method of Ubbink *et al* [9]. Concentrations of serum cobalamin and serum folate were measured by a competitive protein binding method using a SimulTRAC-S radioassay kit (ICN Pharmaceuticals, California, USA). Pyridoxal-5-phosphate (PLP) concentrations in serum were quantified using an HPLC method by Reynolds & Brain [17]. Serum total cholesterol was estimated using an enzymatic CHOD-PAP kit, while serum triglycerides were measured using the Peridochrom GPO-PAP kit (both Boehringer Mannheim). Precipitation for high-density lipoprotein (HDL)-cholesterol estimation employed phosphotungstic Mg^{2+} reagents [18]. All cholesterol assays were carried out on the Cobas Fara autoanalyzer. Low-density lipoprotein (LDL)-cholesterol was estimated using the Friedewald formula:

LDL cholesterol (mmol/L) = total cholesterol – HDL cholesterol – 0.45 × triglyceride.

Methylenetetrahydrofolate reductase (MTHFR) 677C > T genotypes were determined according to the method of Frosst *et al* [19] and have previously been reported [20].

Mean daily energy and nutrient intakes for each subject were calculated from the FFQ using a specialist software package (Q Builder, Tinuviel Software, Warrington).

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Statistical methods

Triglyceride, homocysteine, serum folate, serum cobalamin, and serum pyroxidal phosphate (PLP) were not normally distributed so these were logarithmically transformed. The associations between continuous variables were examined using Pearson correlation coefficients. Comparisons between two groups were made using the Student's independent samples *t*-test. Where necessary, a version of the test appropriate when variances of the samples were unequal was used [21]. For the comparison of more than two groups, for example to test for the effects of MTHFR genotype on thiol concentrations, an one-way ANOVA was used, followed by the Newman-Keuls multiple range comparison test [21]. Multivariable analysis was carried out using general linear modeling. All statistical analyses were performed using SPSS for Windows, version 14.0.

Results

The population descriptive data is presented in Table I. Mean body mass index (BMI) was 26.1 kg/m² showing that 63.7% of the study population was overweight (BMI ≥ 25 kg/m²), with 10.6% being obese (BMI > 30 kg/m²). The mean systolic and diastolic blood pressures (128/80 mmHg) were in the normal range. The mean total plasma cholesterol was 5.83 mmol/L, which is above recommended levels [22]. The dietary intake questionnaire showed that the mean daily intake of fruit and vegetables (2.6 portions) was well below the recommended five portions per day.

The association of thiols with each other and with recognized CVD risk factors is shown in Table II. Cysteine was significantly positively associated with tCys-gly, tHcy, and age. tCys-gly was significantly positively associated with tCys, tHcy, systolic and diastolic blood pressure, and BMI. tHcy was only positively associated with tCys and tCys-gly, and was negatively associated with fruit and vegetable intake.

Associations between thiols and serum B-vitamins and intake are also presented (Table II). tCys was significantly negatively associated with serum vitamin B_{12} and serum B_6, whilst tHcy was significantly negatively associated with serum folate and B_{12}. The only thiol to be associated with B-vitamin intake was tHcy, which was significantly negatively associated with folate and vitamin B_6 intake.

The association between thiol status and categorical CVD risk factors is shown in Table III. Thiols did not differ by smoking status or whether subjects carried out shift work or not (used as a marker of manual/non-manual status). tCys-gly and tCys did not depend on coffee consumption. However tHcy tended to be higher in those who consumed more than 4 cups of coffee per day compared

Table I: Population descriptive data

Variable	Mean (SD)	Median (lower, upper quartile)
Age (years)	39.2 (6.0)	
BMI (kg/m²)	26.1 (3.1)	
Systolic blood pressure (mmHg)	128.1 (14.7)	
Diastolic blood pressure (mmHg)	80.1 (9.5)	
Total cholesterol (mmol/L)	5.83 (1.11)	
HDL cholesterol (mmol/L)	1.09 (0.28)	
LDL cholesterol (mmol/L)	3.90 (0.97)	
Triglycerides (mmol/L)		1.53 (1.05, 2.27)
Portions fruit and vegetables/day	2.57 (1.10)	
tHcy (µmol/L)		7.12 (5.87, 8.62)
tCys (µmol/L)	185.1 (42.2)	
tCys-gly (µmol/L)	30.8 (6.7)	
Folate status (nmol/L)		11.0 (8.5, 14.3)
Vitamin B_{12} status (pmol/L)		261.6 (201.8, 343.5)
Vitamin B_6 status (nmol/L)		33.0 (19.8, 70.2)
Folate intake ((g/day)	306.8 (71.1)	
Vitamin B_{12} intake (µg/day)	10.57 (3.94)	
Vitamin B_6 intake (mg/day)	2.17 (0.43)	
Vitamin B_2 intake (mg/day)	2.33 (0.69)	
% smokers	23.3	
% shift workers	30.6	
% MTHFR TT homozygotes	11.5	

Continuous data presented as mean (SD) except for triglyceride, tHcy, folate, B_{12}, and B_6 status, where data presented as median (lower quartile, upper quartile).

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Table II: Associations between thiols, cardiovascular risk factors and B-vitamin status and intake

	tCys (µmol/L)	tCys-gly (µmol/L)	tHcy (µmol/L)
tCys (µmol/L)	1	0.405** (< 0.001)	0.345** (< 0.001)
tCys-gly (µmol/L)	0.405** (< 0.001)	1	0.264** (< 0.001)
tHcy (µmol/L)	0.345** (< 0.001)	0.264** (< 0.001)	1
Age (years)	0.114** (< 0.01)	–0.018 (0.66)	0.069 (0.09)
BMI (kg/m²)	0.070 (0.08)	0.109** (< 0.01)	–0.016 (0.69)
Systolic BP (mmHg)	0.002 (0.96)	0.081* (0.04)	0.042 (0.30)
Diastolic BP (mmHg)	0.048 (0.23)	0.109** (< 0.01)	0.078 (0.05)
Total cholesterol (mmol/L)	0.057 (0.16)	0.051 (0.20)	0.001 (0.97)
HDL (mmol/L)	0.049 (0.22)	–0.022 (0.58)	0.032 (0.43)
LDL (mmol/L)	0.056 (0.17)	0.024 (0.55)	0.032 (0.42)
Triglycerides (mmol/L)	0.006 (0.87)	0.077 (0.05)	–0.062 (0.12)
Fruit and vegetable intake (portions/day)	–0.017 (0.66)	–0.003 (0.94)	–0.090* (0.025)
Folate status (nmol/L)	0.066 (0.10)	0.044 (0.28)	–0.446** (< 0.001)
B_{12} status (pmol/L)	–0.092* (0.02)	–0.057 (0.16)	–0.377** (< 0.001)
B_6 status (nmol/L)	–0.101* (0.02)	0.025 (0.56)	0.040 (0.35)
Folate intake (µg/day)	0.015 (0.72)	0.013 (0.76)	–0.115** (< 0.01)
B_{12} intake (µg/day)	–0.032 (0.42)	–0.022 (0.58)	–0.037 (0.35)
B_6 intake (mg/day)	0.013 (0.74)	–0.023 (0.57)	–0.127** (< 0.01)
B_2 intake (mg/day)	–0.017 (0.68)	0.010 (0.80)	–0.066 (0.10)

Data presented as Pearson correlation coefficient (p-value). Triglycerides, tHcy and folate, vitamin B_{12} and vitamin B_6 status were logarithmically transformed. *p < 0.05; **p < 0.01. Data presented on n_{max} = 765 subjects.

Table III: Categorical cardiovascular risk factor status, MTHFR status, and thiol status

Variable	tCys (µmol/L)	tCys-gly (µmol/L)	tHcy (µmol/L)
Smoking			
Yes (n = 161)	184.9 (42.3)	30.5 (6.8)	7.26 (6.06, 8.55)
No (n = 462)	185.0 (42.3)	30.9 (6.7)	7.17 (5.85, 8.68)
	P = 0.97	P = 0.47	P = 0.68
Shift work			
Yes (n = 192)	189.5 (45.1)	31.2 (6.8)	7.20 (5.86, 8.70)
No (n = 428)	183.0 (40.9)	30.7 (6.7)	7.20 (5.87, 8.59)
	P = 0.07	P = 0.41	P = 1.00
Coffee consumption/day			
< = *4 cups* (n = 554)	185.0 (42.9)	30.8 (6.9)	7.13 (5.84, 8.54)
> *4 cups* (n = 67)	184.8 (37.2)	30.7 (5.7)	7.72 (6.15, 9.00)
	P = 0.97	P = 0.92	P = 0.08
MTHFR genotype			
+/+ (n = 72)	182.9 (40.7)	30.4 (6.7)	9.46[a] (6.65,12.18)
+/– (n = 273)	185.6 (41.4)	30.9 (6.7)	7.12[b] (5.89,8.64)
–/– (n = 280)	185.2 (43.5)	30.9 (6.8)	6.77[b] (5.75,8.05)
	P = 0.89	P = 0.83	P < 0.001

Data presented as mean (SD) except for tHcy, presented as geometric mean (IQ range). Two group comparisons were made using Students' independent samples *t*-tests, whilst the three genotypes were compared using one way ANOVA with Student-Newman-Keuls post-hoc comparison tests. *p < 0.001 for difference between genotypes, superscript letters show homogeneous subsets.

to those who drank ≤ 4 cups of coffee per day, although this did not reach statistical significance (p = 0.08).

Table III also shows the association between MTHFR 677C > T genotype and thiol status. In univariate analyses, MTHFR 677TT homozygotes did not have altered tCys or tCys-gly concentrations but, as previously reported [18] did have relatively high tHcy concentrations.

The variables described above in univariate analyses were entered into three separate multivariable regression models with tCys, tCys-gly, and tHcy as the outcome vari-

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Table IV: Multivariate regression modeling of thiol status

a) Cysteine

	Average increase in tCys (μmol/L) per unit increase	
	Crude (95% CI)	Adjusted[1] (95% CI)
Age (years)	0.82 (0.26, 1.38)*	0.62 (0.02, 1.21)*
BMI (kg/m²)	0.9 (–0.1, 2.0)	1.0 (–0.1, 2.0)
Vitamin B_{12}[2]	–8.9 (–16.6, –1.2)*	–9.0 (–16.9, –1.1)*
Vitamin B_6[2]	–4.3 (–7.7, –0.8)*	–3.9 (–7.4, –0.5)*

[1]Model contains age, BMI, vitamin B_{12}, and vitamin B_6 status.
[2]On the log scale.
*Significant at 5% level.

b) Cysteinyl-glycine

	Average increase in tCys-gly (μmol/L) per unit increase	
	Crude (95% CI)	Adjusted[1] (95% CI)
Age (years)	–0.02 (–0.11, 0.07)	–0.03 (–0.12, 0.06)
BMI (kg/m²)	0.24 (0.07, 0.40)*	0.2 (0.02, 0.38)*
Systolic BP (mmHg)	0.04 (0.001, 0.07)*	0.02 (–0.02, 0.06)

[1]Model contains age, BMI, and systolic blood pressure.
*Significant at 5% level.

c) Homocysteine

	Unadjusted		Adjusted[1]	
	Average % increase in tHcy per unit increase	Geometric mean (95% CI)	Average % increase in tHcy per unit increase	Adjusted ratio of means (95% CI)
Age (years)	0.4 (–0.1, 0.9)		0.4 (0.0, 0.8)	
BMI (kg/m²)	–0.2 (–1.0, 0.7)		–0.5 (–13.6, 0.3)	
Systolic BP (mmHg)	0.1 (-0.1, –0.3)		0.1 (–0.1, 0.2)	
Serum folate[2]	–30.4 (–34.3, –26.2)*		–24.4 (–28.8, –19.7)*	
Serum vitamin B_{12}[2]	–25.9 (–30.2, –21.3)*		–17.3 (–22.1, –12.2)*	
Serum vitamin B_6[2]	1.0 (–2.0, 4.1)		2.0 (–0.4, 5.1)	
Folate intake	–0.1 (–0.1, 0.0)*		0.0 (–0.1, 0.1)	
Vitamin B_{12} intake	–0.3 (–1.0, 0.3)		0.3 (–0.4, 1.0)	
Vitamin B_6 intake	–9.5 (–15.6, –3.9)*		–6.8 (–16.5, 4.0)	
Riboflavin intake	–3.0 (–6.8, 0.6)		1.0 (–3.0, 6.2)	
Fruit and vegetable intake	–3.0 (–5.4, –0.4)*		–2.7 (–5.2, –0.2)*	
Smoke[3]				
No		7.2 (6.9, 7.4)		1.00 (Ref. Cat.)
Yes		7.3 (6.9, 7.6)		0.97 (0.92, 1.03)
Genotype[3]				
–/–		6.8 (6.6, 7.0)*		1.00 (Ref. Cat.)*
–/+		7.1 (6.9, 7.4)		1.02 (0.97, 1.07)
+/+		9.5 (8.4, 10.7)		1.28 (1.07, 1.40)
Coffee group[3]				
< = 4 cups/day		7.1 (6.9, 7.3)		1.00 (Ref. Cat.)
> 4 cups/day		7.7 (7.1, 8.4)		1.02 (0.94, 1.11)

[1]Model contains age, BMI, systolic blood pressure, smoking, MTHFR genotype, coffee group, folate, vitamin B_{12} and vitamin B_6 status, folate, vitamin B_{12}, vitamin B_6 and riboflavin intake, and fruit and vegetable intake.
[2]On the log scale.
[3]For categorical variables, results presented as geometric mean (95% CI) for unadjusted comparison and adjusted ratio of geometric means. The results are expressed as a percentage increase in (geometric) mean tHcy because the linear regression model for a particular explanatory variable was conducted on the log of tHcy and the coefficients were then back-transformed.
*Comparison significant at 5% level.

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ables; age, BMI, any other significant factors from the univariate analysis, and other variables proposed to be associated with these thiols in previous studies, served as putative explanatory variables (Table IV).

There was a significant increase in tCys of 0.8 µmol/L per year of age ($p = 0.004$). After adjustment for other explanatory variables this was attenuated to 0.6 µmol/L but remained statistically significant ($p = 0.04$). For every unit increase in serum vitamin B_{12} (on the ln scale) there was a significant adjusted reduction in tCys of 9.0 µmol/L, and for serum vitamin B_6 (also on the ln scale) a significant adjusted reduction in tCys of 3.9 µmol/L.

Each unit increase of BMI elevated tCys-gly by 0.24 µmol/L, an effect that remained statistically significant ($p = 0.03$) when attenuated to 0.2 µmol/L after adjustment for other explanatory variables. In contrast, a 0.04 µmol/L elevation in tCys-gly for each unit increase in systolic blood pressure was attenuated and lost significance after adjustment for other explanatory variables.

The main significant continuous variables associated with tHcy concentration after adjustment for other explanatory variables were serum folate (reduction of tHcy by 24.4% for every unit increase in folate on the ln scale), serum vitamin B_{12} (reduction of tHcy by 17.3% for every unit increase in vitamin B_{12} on the ln scale), and fruit and vegetable intake (reduction of tHcy by 2.7% per daily portion of fruit and vegetables). MTHFR 677C > T genotype was the only categorical variable associated with tHcy after adjustment, with the adjusted ratio of geometric means being 1.02 (95% CI 0.97, 1.07) for CT heterozygotes compared to CC homozygotes, and 1.28 (95% CI 1.07, 1.40) for TT homozygotes compared to CC homozygotes.

The addition of other variables to the model for each dependent variable (lipids, dietary intake of B-vitamins, serum B-vitamins, fruit and vegetable intake, blood pressure, MTHFR 677C > T genotype, smoking, and coffee consumption) did not markedly affect the results. The maximum proportion of variation explained for the models described were 7%, 4%, and 34% for tCys, tCys-gly, and tHcy respectively.

Discussion

In this study we investigated the plasma thiol status of 765 healthy men aged 30–49 years, and examined how dietary factors including B-vitamin intake and serum B-vitamins, together with recognized CVD risk factors, were related to thiol status.

Associations between thiols

In univariate analysis the three thiols (tHcy, tCys, and tCys-gly) were significantly associated with one another. This is consistent with their close metabolic connection and in agreement with a number of other cross-sectional studies. In a study of 922 young people aged 4–18 years, Bates *et al* [23], showed that tHcy was strongly and directly correlated with both tCys and tCys-gly, although r-values were not given for the associations, and in an earlier study of approximately 16 000 40- to 67-year-old men and women, El-Khairy *et al* [24] showed an association between tHcy and tCys.

Thiol status and recognized CVD risk factors

In multivariate models, only age was found to be significantly associated with tCys; similarly, only BMI was significantly associated with tCys-gly status. Our findings differ from those of El-Khairy *et al* [24], who found BMI to be strongly associated with tCys, but not with tHcy. Increasing age has previously been associated with relatively higher concentrations of both tCys and tHcy [24–26]. A possible explanation for increased tCys levels is an age-dependent decrease in enzyme activity involved in cysteine and homocysteine metabolism [27], or a reduction in renal function, as thiols have been shown to be elevated in renal disease [28]. Previous studies have also found a link between elevated tHcy and age [29, 30]. The lack of finding of a significant increase in tHcy with increasing age seen in our study may have been due to the relative youth and narrow age range of the subjects (30–49 years), and have arisen previously as a possible consequence of increased atherosclerotic burden in more elderly subjects. Indeed, the El-Khairy study [24] included subjects aged 40–67 years, who are likely to have had significant atherosclerotic disease.

Several large population studies have shown smokers to have higher tHcy than non-smokers [14, 24, 31, 32], although smaller studies, in agreement with this work, have found no such difference between smokers and non-smokers in tCys or tHcy [24, 26, 33–34]. Reasons for the discrepancy in results may simply be due to study size, or related to smoking prevalence or dose in these particular populations.

Although El-Khairy *et al* [24] found BMI to be strongly associated with tCys, no study to our knowledge has previously shown an association between BMI and tCys-gly. However, a recent cross-sectional study in metabolic syndrome patients has shown that patients with individual components of the metabolic syndrome, including increased waist-to-hip ratio, have higher concentrations of gamma glutamyltransferase activity (GGT) and also higher levels of tCys-gly (and tCys) [35]. The action of GGT on glutathione results in its cleavage to tCys and tCys-gly, therefore GGT regulates their circulating concentrations. Our observed association between BMI and tCys-gly may therefore be linked through an increase in GGT. This

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would need to be confirmed through assessment of circulating GGT concentrations, and this analysis was not performed in the current study.

A number of other factors may have affected the demonstrated associations, but were not available for inclusion in these analyses. A number of observational studies have suggested an association between moderate alcohol consumption and tHcy concentrations [32, 36], whilst both renal function (as assessed by serum creatinine) has been shown to affect tHcy, and thiols in general [28, 37]. Inclusion of these factors in our model may have altered the observed associations, and must be accepted as a weakness of these analyses.

Thiols and serum B-vitamins and intake

Inverse relationships were observed between tCys and both serum vitamin B_{12} and B_6, and between tHcy and both serum folate and B_{12} in univariate analyses, and remained significant in multivariate analyses.

Although many studies have shown an association between tHcy and B-vitamin intake and serum B-vitamins [38], few other studies have examined whether other thiols are associated with serum B-vitamins. Bates *et al.* did not show an association between tCys and tCys-gly and B-vitamins in 4- to 18-year-old subjects or those over 65, El-Khairy *et al* did not show an association between these two thiols and B-vitamin intake, and Midttun *et al.* found no relationship between serum PLP and tCys concentrations [23, 24, 39]. In this study, we observed an independent inverse association between tCys and plasma B_6 and B_{12}. A higher concentration of vitamin B_{12} may lead to reduced tCys due to improved tHcy remethylation. In contrast to the inverse relationship observed in this study, other studies have found no relationship between PLP and tCys concentration [23, 24, 39, 40], however we were unable to adjust for serum creatinine in this study. A higher concentration of vitamin B_6 might be expected to lead to increased trans-sulfuration and so increased tCys, however, PLP-dependent enzymes are involved in the catabolism as well as the formation of cysteine [41, 42]. It is possible that a higher vitamin B_6 status leads to increased formation of cysteine but also increased catabolism. More research is needed to fully elucidate the effects of vitamin B_6 status on cysteine metabolism.

Folate and B_6 intake were significantly inversely associated with tHcy status in univariate analyses, but this relationship lost significance after multivariate adjustment. In contrast, fruit and vegetable intake, which also was inversely associated with tHcy concentrations, remained significant in the adjusted model. The latter is in accord with other studies [43, 44], and it has been proposed that plasma folate is a biomarker of fruit and vegetable consumption [45]. In addition, two intervention studies have shown that increasing fruit and vegetable consumption lowers tHcy concentrations [46, 47].

MTHFR genotype and thiol status

In both uni- and multivariate analyses, MTHFR 677C > T genotype was only associated with tHcy status, and this has been reported previously for this data set [20]. A number of other studies have examined MTHFR 677C > T genotype in relation to tCys status in renal patients, but to our knowledge similar analyses have not previously been undertaken in healthy volunteers. Kimura *et al* [48] reported that the MTHFR TT genotype was associated with low tCys levels in hemodialysis patients, although this is contrary to what might be expected from a knowledge of the metabolic pathway. In contrast, Marcucci *et al* [49] did not report any difference in tCys status by MTHFR 677C > T genotype in renal transplant recipients.

In conclusion, this study has shown that in this relatively young, healthy Northern Irish population, tCys increases with increasing age, whilst tCys-gly increases with increasing BMI. Apart from these findings, significant relationships between either tCys or tHcy and other CVD risk factors were not observed. As expected, significant associations were observed between tHcy and both serum B vitamins and MTHFR 677C > T genotype and tHcy. Independent associations were also demonstrated in multivariate analyses between tCys and both serum vitamin B_{12} and B_6, and between tHcy and fruit and vegetable intake. Overall the explanatory variables identified in this study accounted for a modest amount of tCys and tCys-gly variation (7% and 4%, respectively), and a relatively large proportion of tHcy variation (34%). There has been extensive investigation of the role of tHcy in CVD but little concerning tCys. Due to their biochemical similarities and metabolic connections, further studies of tHcy and tCys in larger groups are needed to confirm the above results and, in particular, to further evaluate the suggested association of tCys with the development of CVD.

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